REMARKS

The present application is directed to methods for treating viable mammalian transplant tissue in such a manner as to temporarily ablate MHC Class I antigen complexes from the surface of the transplant tissue, thereby rendering the viable tissue suitable for transplantation into a host. After the transplanted donor tissue becomes established in the host (recipient), and after avoiding initial immune attack by killer T cells, the continuing expression of MHC Class I molecules and the returning presentation at the surface of MHC Class I antigen complexes, provides the normal mechanism for educating the host's immune system to identify the new (donor) tissue as "self", leading to deletion of the population of natural killer T cells capable of recognizing and causing the rejection of the donor tissue. The claims have been previously amended to emphasize the timely (and temporary) ablation of MHC Class I surface antigens and use of the donor tissue in transplantation before the reappearance of the Class I antigens on the donor tissue surface.

In response to the first Office Action, Applicant amended Claims 1 and 12 to specifically recite two critical features of the claimed invention, namely that the donor tissues are viable at the time of transplantation (and remain viable in the host) and that the donor tissues are transplanted into the host prior to the reemergence of MHC Class I antigens on the surface of the treated tissue.

35 U.S.C. §102(b)

In the Office Action of September 24, 2003, the Examiner has maintained the rejection of Claims 1-3 and 5-8 under 35 U.S.C. §102 as being anticipated by Oliver et al., U.S. Pat. No. 4,399,123 ("the '123 patent"). With respect to the '123 patent, the Examiner states,

> "The ['123 patent] is considered to anticipate the claimed invention because it teaches identical method for inhibiting transplant rejection comprising identical active steps of treating and transplanting donor tissues and identical structural elements including two enzymatic treatments and various combinations of donor tissues and host organisms as the claimed method." (See, final Office Action at page 5.)

Oliver et al. disclose a method for treating fibrous tissue first with a proteolytic enzyme such as trypsin to remove antigenic nonfibrous tissue proteins, followed by treatment of the tissue with a carbohydrate-splitting enzyme to remove polysaccharides, mucopolysaccharides, and glycoproteins, which may or may not be antigenic (see, col. 3, lines 8-29), followed by treatment of the tissue with a stabilizer cross-linking agent such as glutaraldehyde. (See, e.g., col. 4, lines

38-48.) When the Oliver et al. patent is analyzed in detail, it is obvious that the teachings of this patent and the present invention are vastly different and unrelated.

Applicant respectfully disagrees with the Examiner's assertion that the '123 patent teaches an identical method for inhibiting transplant rejection to the present application. There are in fact significant differences between the two disclosures. First, and most importantly, the treatment disclosed in the '123 patent results in the grafting of non-viable fibrous tissue. In contrast, the disclosure of the present application teaches a method for inhibiting transplant rejection by implanting live, viable donor tissue in a recipient. The Examiner's attention is directed to Examples 1, 2, 3, 4, and 6 of the '123 patent. As is disclosed in all these examples, the initial tissue enzyme treatment (e.g., trypsin) according to the '123 patent includes incubation of the tissue with 0.5mg/ml sodium azide for 28 days. (See, for example, col. 6, lines 46-50.) Applicant asserts that it is well known in the art that sodium azide treatment, even at concentrations much lower than taught in the '123 patent, is almost immediately lethal to all eukaryotic cells and, as such, the treatment of tissue for 28 days with this chemical, which is taught as an essential step in the '123 patent transplantation process, would most assuredly result in the death of all the cells of the donor tissue sample. Expression and reassertion of any of the surface molecules normally present on the transplant tissue would be impossible, following the teachings of the '123 patent. Thus, the teachings of the '123 patent cannot anticipate Applicant's claims, which call for transplantation of viable tissue.

In the final Office Action, the Examiner states that the arguments presented by Applicant regarding the immediate death of eukaryotic cells treated with sodium azide were found unpersuasive. According to the Examiner,

> "However, upon review of the teaching of the ['123 patent] is it [sic, it is] established that the ['123 patent] *does not disclose* that the donor tissue is rendered dead or non-viable as the result of treatment before transplantation. [The '123 patent] clearly teaches that sodium azide is used as 'bactericide' . . . which is reasonably expected to achieve elimination of a possible pathogen transmission during transplantation *rather than to render the donor tissue dead or non-viable*". (See, final Office Action, page 4, emphasis added.)

The stated intention of Oliver et al. to kill bacteria does not change the FACT that all viable cells associated with the transplant tissue are killed by the required sodium azide treatment. The Examiner cannot ignore this fact of biology merely because her chosen references are silent about it. Applicant asserts that the reason the '123 patent does not specifically state that the

sodium azide treatment leaves the transplant tissue non-viable is because the viability of the treated and sterilized fibrous tissue is of no moment to the '123 invention: (1) it is immediately evident to one skilled in the art of transplantation that the transplant tissue utilized in the '123 patent is non-viable, and (2) the '123 patent seeks to take advantage of the mechanical strength of purified fibrous tissue and its inherent structural characteristics that allow it to be infiltrated and colonized by viable host cells, making it suitable as a temporary or permanent matrix for the repair of tissue damage regardless of tissue viability. The '123 patent therefore does not require (or teach) the transplantation of viable tissue (as in Applicant's invention).

Because this point remains in dispute, Applicant has performed experiments to show the results of following the teachings of Oliver et al. In order to demonstrate to the Examiner that treatment of viable cells according to the '123 patent, i.e., in 0.5mg/ml sodium azide for 28 days would, without question, result in 100% elimination of viable cells, Applicant submits herewith the Declaration of the inventor, Dr. Denise L. Faustman, M.D., Ph.D., pursuant to 37 C.F.R. §1.132 (Tab A). The Declaration of Dr. Faustman includes a set of experiments demonstrating the treatment of various human- and murine-derived eukaryotic cells with varying concentrations of sodium azide ranging from 0 to 0.5mg/ml (the latter, and highest concentration tested, 0.5mg/ml, being the concentration disclosed for treating tissues prior to transplantation in the '123 patent). Specifically, Dr. Faustman treated murine T cells isolated from splenocytes, kidney cells, and liver cells, and also human peripheral blood lymphocytes (PBLs), with 0, 0.001, 0.002, 0.01, 0.02, 0.10, and 0.5mg/ml sodium azide. Results of the treatments are shown in Tables 1A, 1B, and 1C included in the Declaration. As seen in Table 1A, the viability of T cells isolated from mouse splenocytes was adversely affected within 22 hours by treatment in sodium azide concentrations as low as .001mg/ml, i.e., after a short treatment with a sodium azide concentration that is **500 times lower** than taught in the '123 patent. And, as seen in Table 1A, 100% of the cells treated with sodium azide concentrations as low as 0.001mg/ml were non-viable within three days. The same results are seen with murine kidney and liver cells treated with the sodium azide concentrations of 0.001 mg/ml – 0.5 mg/ml (see, Declaration, Table 1B), and human PBLs treated with the same sodium azide concentrations (see, Declaration, Table 1C). All cells were dead within 3 days of treatment regardless of the sodium azide concentration (except for control cells: 0 mg/ml sodium azide).

A further indication that the '123 patent does not teach the use of viable tissue for transplant is seen by noting that in Examples 1, 2, 3, 4, and 6 of the '123 patent, the fibrotic tissue treatment also includes, subsequent to the 28 day sodium azide treatment, a glutaraldehyde

crosslinking step, and in Example 5, transplant tissue is treated with 0.1% – 0.5% formaldehyde. However, as with sodium azide, Applicant asserts that glutaraldehyde or formaldehyde treatment also would be understood in the art to be lethal to viable transplant tissue.

As this point also remains in dispute, Applicant has performed additional experiments to show the results of following the teachings of Oliver et al. to demonstrate that treatment with aldehyde cross-linkers is lethal to viable transplant tissue. Included in Applicant's Rule 132 Declaration submitted herewith are the results of experiments performed by Dr. Faustman that demonstrate the lethality of treatment with an aldehyde cross-linking agent, in particular, formaldehyde (as taught in Example 5 of the '123 patent). As stated in the Declaration, Dr. Faustman conducted experiments incubating various human- and murine-derived cell types in varying concentrations of formaldehyde for 5 minutes, followed by an assessment of cell viability at 22 hours and 3 days post-formaldehyde treatment. Specifically, Dr. Faustman treated T cells isolated from murine NOD and B6 splenocytes, kidney cells, and liver cells and human peripheral blood lymphocytes (PBLs) in 0, 0.1, 0.2, and 5% formaldehyde. As seen in Table 2A, after treatment for 5 minutes in formaldehyde concentrations as low as 0.1%, all cells were dead 3 days after treatment for all tested formaldehyde concentrations, even 0.1%. The same results are seen with murine kidney and liver cells (Table 2B) treated with the same formaldehyde concentrations and with human PBLs also treated with the same formaldehyde concentrations (Table 2C): After treatment for 5 minutes in formaldehyde concentrations as low as 0.1%, all cells were dead within 3 days after treatment.

These experiments graphically illustrate what is known, as a matter of law, by the hypothetical person of ordinary skill in the art, i.e., that the pre-transplant treatments of Oliver et al. render the donor tissue non-viable (regardless of whether such non-viability is belabored in the '123 patent specification or not). This being the reality of the '123 patent teachings, the '123 patent cannot be considered to teach Applicant's invention, calling for transplantation of viable tissue.

With respect to Applicant's assertion in the response to the first Office Action that the glutaraldehyde treatment disclosed in the '123 patent is also lethal to eukaryotic cells, the Examiner replied,

> "[The '123 patent] clearly teaches that the cross-linking with glutaraldehyde is intended for removal of antigenicity in the donor tissue . . . and, thus this treatment is intended for inhibiting rejection by host mammal of donor tissue from another mammal as encompassed by the claimed method. Although it could be true that the viability of the donor tissue might have been

> reduced or 'temporarily' reduced by the glutaraldehyde cross-linking, the cross-linked tissue remains viable at least to some extend [sic, extent]." (See, final Office Action, pages 4-5.)

First, by placing the term "temporarily" in quotation marks, the Examiner appears to be stating that glutaraldehyde treatment only temporarily effects the viability of the cells and is then somehow attempting to equate that statement with "temporary" as recited in Claim 1 of the present application. In this instance, the Examiner is mistaken for two reasons, (1) glutaraldehyde treatment does not "temporarily" reduce the viability of a cell, it kills the cell permanently, and (2) the present invention is not related to "temporarily" reducing the viability of any cells, it is directed to "temporarily" removing MHC Class I surface antigens from viable donor tissue, and the Applicant's claims specifically require that the transplant tissue remains viable.

Second, with respect to glutaraldehyde treatment, as with the sodium azide treatment discussed above, the Examiner seems to be implying that unless the reference overtly states that the glutaraldehyde treatment is lethal to cells, the Examiner may conclude (against reality) that glutaraldehyde treatment must not be lethal to eukaryotic cells. Applicant respectfully submits that transplant tissue subjected to 16 hours of glutaraldehyde treatment is inherently non-viable, that such absence of viability would be apparent to anyone of ordinary skill in the art, and that the silence of the '123 patent with respect to stating this inherent and necessary FACT of biology does not allow the patent to teach the opposite of what it teaches.

In support of her interpretation of the '123 patent, the Examiner has cited Pathak et al., U.S. Pat. No. 6,322,593, Method For Treating Cross-Linked Biological Tissues, and the abstract of Gallagher et al., Studies on the accessory requirement for T lymphocyte activation by concanavalin A, *Clinical and Experimental Immunology*, 66(1): 118-125 (1986).

Pathak et al. actually provides clear support for Applicant's assertion that glutaraldehyde treatment is lethal to eukaryotic cells. The purpose of the Pathak et al. reference is to reduce the toxic effect seen with aldehyde treated tissues, particularly in the field of implantable bioprosthetic devices,

> "An important issue when using polyfunctional aldehydes for treating biological tissues relates to the toxicity of the resulting cross-linked material." (See, Pathak et al., column 2, lines 1-3.)

The Examiner's attention is directed to Table 1 of the Pathak et al. reference beginning at column 6, line 30. Table 1 shows the percent viability of mouse cells following treatment with glutaraldehyde alone or glutaraldehyde plus sodium bisulfite. As seen in Table 1, treatment of

mouse cells in 0.25% glutaraldehyde for 5 hours or 16 hours resulted in **80%** and **93%** cell death, respectively.

In fact, from these studies, Pathak et al. conclude,

> "From Table 1, it is clear that the viability of BALB-3T3 cell is severely compromised by tissue samples treated with glutaraldehyde for either 5 hours or 16 hours . . ." (See, column 6, lines 47-50, emphasis added.)

Therefore, it can be expected from Pathak et al. that the 16-hour glutaraldehyde treatment disclosed in the '123 patent would result in production of non-viable tissue for transplant, contradicting the Examiner's interpretation.

The Gallagher et al. reference (a full copy of which is provided herein at Tab B) studies the ability of accessory cells (AC) to supply signals for the induction of T cell proliferation. (See, page 119, 1st full paragraph.) One method was to treat accessory cells in 0.1% glutaraldehyde for 10 seconds, then evaluate their ability to assist in T cell proliferation. Gallagher et al. concluded that AC cells mildly treated with glutaraldehyde could not induce T cell proliferation:

> "Examination of the cultures throughout incubation showed that aggregation of the cells in culture was intimately associated with proliferation. Cycloheximide-treated AC were capable of inducing both aggregation and proliferation, while [glutaraldehyde] fixed cells could achieve neither." (See, page 124, 3rd paragraph).

Applicant asserts that Gallagher et al.'s teaching that AC cells survived incubation for 10 seconds with 0.1% glutaraldehyde does nothing to negate the fact that it is well known in the art that glutaraldehyde treatment is lethal to viable cells and does nothing to negate the fact that the 16 hours of glutaraldehyde treatment taught by the '123 patent is lethal to transplant tissue. The Gallagher et al. reference is a direct teaching to the art that any glutaraldehyde treatment of living cells should be quick (i.e., lasting only seconds), and mild (i.e., 0.1% glutaraldehyde); but even then, such a treatment can still have a profound affect on the functionality of the cell, as evidenced by the inability of treated AC cells to carry out their normal function of assisting in the stimulation of T cell proliferation as a result of this glutaraldehyde treatment.

Because the Examiner relied solely on the abstract of Gallagher et al., Applicant provides herewith the full journal article from which the abstract was derived (Tab B).

With respect to Applicant's earlier remarks distinguishing the teachings of the '123 patent from the present invention the Examiner responds,

> "Applicant also argues that the goal of the cited ['123] patent . . . is a retention of matrix structure of the donor tissue rather than stimulation of tolerance by the host of the donor tissue after transplantation as encompassed by the applicants' invention . . . However, it is noted that the concept of 'tolerance' or of the host immune system 're-education' is not within the invention as claimed. Further, although the applicant's generic disclosure might suggests [sic] this concept as a possible mechanism of action . . . it is not supported by the exemplified disclosure (examples 1-4)." (See, final Office Action, page 5).

First, Applicant did not argue that the goal of the '123 patent was the retention of the matrix structure rather than stimulation of tolerance. Applicant stated that, as disclosed in the '123 patent, one feature of the Oliver et al. teaching was that the fibrous tissue, after harsh chemical treatment, could still provide a "scaffold-like" matrix, i.e., a porous structure, to allow cellular in-growth and over-growth of the fibrous tissue at the graft site:

> "This fibrous tissue preparation is capable of being infiltrated and colonized by the host cells of another individual . . . revascularized and, in some cases reepidermilized to form a permanent repair." (See, col. 2, lines 24-29, emphasis added.)
>
> * * *
>
> "In cutaneous wounds the implanted purified fibrous tissue becomes overgrown with epidermis provided it is covered by a suitable dressing and eventually *assumes the appearance* of normal skin." (See, col. 5, lines 40, emphasis added.)

Second, Applicant never "argued" that the '123 patent did not seek to "stimulate tolerance by the host of the donor tissue". Rather, Applicant stated a fact, namely, that the '123 patent did not disclose or suggest the temporary removal of one specific type of surface protein, i.e., MHC Class I surface antigens, from viable mammalian donor tissue to facilitate successful transplantation of the live tissue into a recipient. (See, response to first Office Action, page 12.) In the response, Applicant further distinguished the present invention from the '123 patent by pointing out that the '123 patent described and claimed a treatment of fibrotic tissue to strip proteins and antigenic molecules, to remove structures associated with the tissue (for example sebaceous glands), and to sterilize the tissue, and then to take advantage of its fibrous (mechanical) characteristics, namely, to provide a permanent or temporary sterile dressing or repair for a tissue wound. This is not an "argument" but the invention as claimed in the '123 patent:

Claim 1: "A fibrous tissue preparation of mammalian dermal origin which is suitable for homo or heterotransplantation for the permanent repair of or as temporary dressing for cutaneous wounds and soft tissue injuries, which preparation is substantially free of nonfibrous tissue proteins and antigenic polysaccharides, mucopolysaccharides, and glycoproteins."

Applicant further pointed out that because of the sterilization process taught by Oliver et al., such fibrous tissue must inherently be non-viable, and therefore re-expression of surface antigens (as required by Applicant's claims) is not possible following the teachings of Oliver et al.

Third, in contrast to the Examiner's assertion, "the concept of 'tolerance' or of the host immune system 're-education'" is within the invention as claimed. For example, Claim 1 is directed to:

Claim 1: "A method for inhibiting rejection by a host mammal of donor tissue from another mammal which is transplanted into the host mammal, said method comprising,
(a) treating viable donor tissue with an enzyme effective for temporarily ablating MHC Class I antigens from said donor tissue,
(b) transplanting said treated, viable donor tissue into said host mammal before MHC Class I antigens are re-expressed on the surface of said donor tissue, and
(c) maintaining said viable donor tissue in said host."

According to the method as claimed, the temporary removal of MHC Class I antigens, the transplantation before MHC Class I re-expression, and the successful maintenance of the viable tissue within the host, necessarily defines a method, the steps of which result in host "tolerance" of donor tissue. Reciting "re-expression" of the antigenic structures coupled with maintenance of the donor tissue in Claim 1 directly conveys the concept of "re-education" of the host immune system.

Fourth, with respect to the Examiner's assertion that "this mechanism of action is not supported by the exemplified disclosure", the Examiner's attention is directed to Example 2 beginning on page 9 of the specification. In Example 2, rat hepatoma cells were treated with papain to remove MHC Class I surface antigens, then transplanted under the kidney capsule of non-immunosuppressed B6 mice. Removal and analysis of the transplanted cells demonstrated that untreated rat cells were rapidly rejected within 3 to 5 days after transplantation, whereas 9 of

the 10 mice that received donor cells treated according to the present invention had viable rat hepatoma cells under the kidney capsule up to 30 days post-transplantation.

This is a clear demonstration that the present application teaches: (1) that cells can be treated to specifically remove MHC Class I surface antigens and retain viability; (2) the treated cells will remain viable after transplantation into a host; (3) the host will not recognize the donor cells as immunologically foreign; and (4) the host will not mount an immune response against the donor tissue treated in accordance with the invention. In other words, Applicant has demonstrated the development of tolerance by the host for the donor tissue, which requires the host immune system to be "re-educated" to recognize the re-expressed MHC class I surface antigens as "self" molecules

The '123 patent does not disclose or suggest the temporary removal of MHC Class I surface proteins specifically from viable mammalian donor tissue to facilitate successful transplantation of the viable tissue to a recipient. Accordingly, the '123 patent is insufficient to anticipate any of the pending claims under 35 U.S.C. §102.

35 U.S.C. §102(b)

The Examiner has also maintained the rejection of Claims 1-3, 5-7, 9, and 12 under 35 U.S.C. §102 as being anticipated by Oliver et al., U.S. Pat. No. 5,397,353 ("the '353 patent").

With respect to the '353 patent the Examiner states,

> "The ['353 patent] is considered to anticipate the claimed invention because it teaches an identical method for inhibiting transplant rejection comprising identical active steps of papain enzymatic treatment of donor tissues and two steps of transplanting." (See, final Office Action, page 7).

Applicant asserts that the teaching of the '353 patent, like the '123 patent, is not related to a treatment for the temporary ablation of MHC Class I antigens on the surface of viable donor tissue for transplantation to a mammalian host and therefore, in contrast to the Examiner's assertion, does not teach an identical method for inhibiting transplant rejection. Similar to the '123 patent, the '353 patent also teaches a treatment that leads to the transplantation of non-viable fibrotic tissue. As seen in the '353 patent working examples, the fibrotic tissue undergoes an even harsher chemical treatment than the tissue of the '123 patent as the initial tissue treatment step is for at least one hour in acetone followed by treatment in 0.5 mg/ml sodium azide for 28 days, then a nine-hour incubation in a 0.1% solution of hexane diisocyanate as a crosslinking agent. Therefore, the tissue treatment disclosed in the '353 patent, i.e., involving treatment with acetone

and sodium azide and crosslinking agents, would have the same lethal effect on the donor tissue as discussed above in relation to the teaching of the '123 patent. Thus, because Applicant's method requires viable donor tissue, the '353 patent teaching cannot anticipate the present invention.

Moreover, Applicant points out that it would also be appreciated by a person of ordinary skill in the art that treatment with acetone as taught in the '353 patent is also lethal to viable transplant tissue. To demonstrate this, Applicant refers again to the Declaration of Denise L. Faustman, M.D., Ph.D. pursuant to 37 C.F.R. §1.132 submitted herewith, demonstrating that treatment of cells with acetone for 1 hour leads to 100% cell death in less than one day. Applicant treated murine NOD and B6 T cells isolated from splenocytes, kidney cells, and liver cells, and human peripheral blood lymphocytes (PBLs) with or without acetone for one hour. As seen in Table 3A of the Declaration, treatment of murine T cells from splenocytes in acetone for 1 hour results in the death of 100% of the cells within 22 hours after treatment. The same results are seen with similarly treated kidney and liver cells (see, Declaration, Table 3B) and human peripheral blood lymphocytes (see, Declaration, Table 3C), i.e., all cells died within 22 hours after treatment in acetone for 1 hour.

As stated previously, like the '123 patent, the purpose of the method of the '353 patent is to strip virtually all viable cells from the tissue and to destroy virtually all the cellular elements associated with the dermal fibrotic tissue prior to implantation of the tissue:

> "In accordance with one aspect of the present invention there is provided a substantially non-antigenic fibrous tissue preparation . . . which preparation is substantially free of non-fibrous tissue proteins and glycoproteins, is non-cytotoxic, substantially free of cellular elements, and substantially free of lipids and lipid residues . . . Those substances said to be '*substantially free*' of materials generally contain less than 5% of and preferably less than 1% of said materials." (See, col. 3, lines 15-42, of the '353 patent, emphasis added.)

Also, like the '123 patent, the '353 patent seeks to take advantage of the structural characteristics of fibrotic tissue that make it useful as a growth substrate for host cells:

> "It is a further advantage that in this process the original architecture of the collagenous fibrous material is preserved. The material is neither solubilized or denatured in the process so it natural structure is maintained which makes an implant derived from the material feel natural . . ." (See, col. 3, lines 7-12, emphasis added.)

* * *

> "When implanted subcutaneously the new collagenous sheet material became recolonized by host cells and revascularized . . . Furthermore, in tissue culture the new material was found to become covered with a structural epidermis . . . and when implanted into skin wounds it became overgrown with epidermis and eventually appeared as normal skin apart from the *absence of hairs*." (See, col. 5, lines 10-22, emphasis added.)

Therefore, as with the '123 patent, the '353 patent teaches the grafting of non-viable, sterile, fibrous tissue to take advantage of the structural or "architectural" characteristics that remain after harsh enzyme and chemical treatment.

In addition, not only does the '353 patent seek to take advantage of the structural characteristics of sterilized and completely denuded fibrotic tissue, Claim 1 specifically recites the characteristics retained by the treated tissue as well as the elements that have been stripped away, i.e., Claim 1 describes a structurally intact, but dead fibrotic tissue,

> "1. A non-resorbable, substantially non-antigenic collagenous fibrous tissue preparation of human or animal tissue origin, which is suitable for homo- or heterotransplantation as a permanent repair for cutaneous wounds and soft tissue injuries, which preparation retains the natural structure and original architecture of said human or animal tissue, is substantially free of non-fibrous tissue proteins and glycoproteins, is substantially free of cellular elements, is substantially free of lipids and lipid residues and is non-cytotoxic, wherein said preparation is capable when implanted of being recolonized by host cells and revascularized while being resistant to calcification." (See, col. 7, line 24 to col. 8, line 9, emphasis added.)

Therefore, the '353 patent does not teach or suggest each and every element of Applicant's claimed invention, and in no way communicates to the skilled practitioner a treatment for viable tissue calling for temporary ablation of MHC Class I antigens from the surface of donor tissue *which leaves the ability of the tissue to regenerate MHC Class I antigens intact*. As with the '123 patent, the treatment disclosed in the '353 patent results in the permanent removal of all surface proteins from donor tissue which is no longer viable and, as such, would be unsuitable for practicing the novel method disclosed in the present application.

In addition, with reference to the '353 patent, the Examiner concludes,

> "Thus, the method for inhibiting transplant rejection of [the '353 patent] results in ablating, at least temporarily, of the immune-mediated attack by the host's immune system within the scope of the presently claimed invention." (See, final Office Action, page 7).

Again, this is similar to the Examiner's assertion made relating to the teaching of the '123 patent. As stated above, the present invention is not related to temporarily "ablating" the immune-mediated attack of donor tissue; rather, the present invention seeks the permanent tolerance of donor tissue by a host via the method of (1) temporarily ablating key surface molecules from the donor tissue that would otherwise signal the immune system to attack the donor tissue, while (2) retaining the viability of the donor tissue so as to permit re-establishment of the ablated surface molecules and contribute to re-education of the host's immune system *in situ* to tolerate the non-endogenous donor tissue.

With respect to Applicant's earlier remarks distinguishing the teachings of the '353 patent from the present invention, the Examiner responds,

> "Upon review of the teaching of the ['353] patent . . . is it [sic, it is] established that the cited ['353] patent . . . does not disclose that the donor tissue is rendered dead or non-viable as the result of treatment before transplantation. The sodium azide is used as 'bactericide' . . . but not to render the donor tissue dead or non-viable . . . Therefore, the donor tissue in the method of the ['353] patent . . . is not dead and/or it is considered to be 'viable' or capable either re-establish expression of surface antigen after transplantation or to re-educate the host immune system in order to inhibit the transplant rejection within the meaning of the claims as argued." (See, final Office Action, page 8, emphasis added.)

Again, Applicant points out that the failure of the '353 patent to characterize the treated fibrous tissue as non-viable does not amount to a teaching that it is viable and thus relevant to Applicant's invention. Applicant has proved that following the teaching of Oliver et al. leaves no viable cells intact, and accordingly neither the '353 patent nor the '123 patent teaches Applicant's invention. For this reason, reconsideration and withdrawal of the rejections under 35 U.S.C. §102 are in order.

35 U.S.C. §103

The Examiner has maintained the rejection of Claims 1-9 and 12-23 under 35 U.S.C. §103 as being unpatentable over Oliver et al., U.S. Pat. No. 4,399,123 and Oliver et al., U.S. Pat. No. 5,397,353 taken with Galati et al., *Cytometry*, 27: 77-83 (1997).

For the reasons set forth above, the teachings of the '123 patent and the '353 patent are unrelated to the novel methods taught in the present application.

With respect to Galati et al., the Examiner states,

> [Galati et al.] clearly and particularly demonstrates that papain removes MHC Class I molecules of glycoprotein nature from cell surface (abstract) and it teaches that the MHC class I glycoproteins are expressed on nearly all nucleated cells . . . It also teaches that other than the MHC class I surface associated molecules remain unaffected by papain digestion . . ." (See, final Office Action, page 10.)

Applicant asserts that the Galati et al. reference, when considered either alone or in combination with the '123 and/or '353 patents, neither discloses nor suggests the invention disclosed in the present application. Galati et al. report a method for quantitating MHC Class I expression by flow cytometry. Essentially, Galati et al. sought an alternative to quantitating membrane molecules by the (then) traditional method of analyzing surface-bound antibodies by employing "a combined cytometric/HPLC procedure" (see page 81, col. 2). According to this reference,

> "We relied on the well-described property of the enzyme papain to cleave the extracellular hydrophilic portion of MHC complexes from crude membrane preparations . . . We speculated that papain digestion of living cells could result in a quantitative decrease of the MHC-associated membrane fluorescence . . . Our speculation indeed proved correct, and soluble MHC molecules could easily be quantitated by HPLC analysis . . ." (See, Galati et al. at page 81, col. 2, to page 82, col. 1.)

Therefore, Galati et al. report that isolated (cell free) MHC molecules can be quantitated after treatment of living cells with the enzyme papain. Galati et al. do not disclose or suggest anything with regard to the papain-treated cells. More specifically, Galati et al. do not teach that the cells remain viable, or that they are suitable for use in transplantation. Most importantly, there is no suggestion in the Galati et al. reference that after papain treatment the cells would be protected from immune recognition by the host if used as transplant tissue, nor any suggestion that if maintained in the host, the host's immune system would become tolerant of the cells (i.e., as a function of the renewed expression of MHC Class I antigen complexes by the transplanted cells). No mention of transplantation is made by Galati et al.

The combination of the '123 or '353 patents and Galati et al. do not suggest a method for inhibiting transplant rejection by treating viable donor tissue to temporarily ablate MHC Class I surface antigens and then transplanting the treated, viable tissue into a host. The combined teachings still guide the person of ordinary skill in the art to produce non-viable tissue before

transplantation. Accordingly, the combination of the '123 or '353 patents and Galati et al. is insufficient to render the present claims obvious within the meaning of 35 U.S.C. §103. Reconsideration and withdrawal of the rejection are respectfully requested.

In response to Applicant's earlier remarks distinguishing the Galati et al. reference from the present invention, the Examiner replies,

> "[A]pplicant argues that the reference by Galati does not mention a transplantation of papain treated tissue . . . However, this reference is relied upon to demonstrate that the papain enzyme removes antigenic structures that are the MHC Class I antigens as encompassed by the claimed invention. The transplantation of the papain treated donor tissues is taught by the cited patents ['123] and ['353] combined." (See, final Office Action, pages 11-12.)

However, Applicant asserts that the combination of the '123 and '353 patents and Galati et al. does not suggest *a method for inhibiting transplant rejection by treating viable donor tissue to temporarily ablate MHC Class I surface antigens* and then transplanting the treated, *viable* tissue into a host. At most, the '123 and '353 patents teach that a sterilized fibrous tissue completely stripped of all surface molecules and completely and permanently devoid of all cellular function is suitable as a transplantable scaffold for host cell infiltration leading to wound repair. As demonstrated above, that is what is disclosed and claimed in the '123 and '353 patents. Applicant asserts that the Examiner overlooks the fact that the '123 and '353 patents both teach treatment of tissue with a series of lethal chemicals as the only way to practice their methods. It is improper for the Examiner to zero in on one chemical that the references and the present invention have in common (papain), and then assert that the methods are identical while ignoring the rest of the disclosed teachings that are critical to the references' teachings, viz., the use of deadly chemicals not suitable for use in the method of the present invention. In addition, the inclusion by the Examiner of Galati et al. in the combination does nothing to bridge the gap between the teachings of the '123 and '353 patents and the present invention. The difference between the combined teachings of the cited prior art and Applicant's invention is still that the combined prior art teachings result in non-viable transplant tissue, whereas the Applicant's claims require viable transplant tissue. Accordingly, the combination of the '123 or '353 patents and Galati et al. is insufficient to render the present claims obvious within the meaning of 35 U.S.C. §103.

Reconsideration and withdrawal of the rejection are respectfully requested.

35 U.S.C. §103

The Examiner has maintained the rejection of Claims 10 and 11 under 35 U.S.C. §103 as being unpatentable over Oliver et al., U.S. Pat. No. 4,399,123 and Oliver et al., U.S. Pat. No. 5,397,353 taken with Galati et al., *supra*, all as applied to Claims 1-9 and 12-14 as stated above, and further in view of Stone et al., *Transplantation*, 65: 1577-1583 (1998).

For the same reasons set forth above, the '123 and '353 patents, and the Galati et al. references, considered either alone or in combination with Stone et al., do not teach or suggest the invention disclosed in the present application for inhibiting rejection of viable transplant tissue by temporarily ablating MHC Class I antigens from the surface of viable donor tissue prior to transplantation.

With respect to Stone et al., the Examiner states,

> "Stone et al. . . . discloses a method for inhibiting transplant wherein the method comprises step of treating donor tissue with galactosidase and step of transplanting the treated tissue into host recipient and wherein the method results in a reduction of inflammatory reaction or immune response of recipient host." (See, final Office Action, page 13.)

Stone et al. report on the effect of eliminating α-gal epitopes from porcine articular cartilage by incubation with α-galactosidase followed by implantation into the suprapatellar pouch of cynomolgus monkeys and monitoring of the immune response. (See, Stone et al., page 1578, right column, 1st paragraph.)

According to Stone et al.,

> "This study shows that treatment of cartilage with α-galactosidase can successfully prevent anti-Gal immune response against the xenograft. However, the primate immune system reacts against cartilage-specific antigens, resulting in antibody formation as well as macrophage-mediated chronic inflammatory reaction in some of the xenografts." (See, page 1578, right column, 1st paragraph.)

Stone et al. conclude,

> "Overall, the present study indicates that the enzymatic removal of α-gal epitopes from cartilage xenograft is an important first step in decreasing the immune rejection in primates." (See, page 1583, left column, last paragraph, emphasis added.)

Stone et al. study cynomolgus monkeys transplanted with porcine cartilaginous tissue pretreated with α-galactosidase to permanently remove α-gal epitopes from the surface of the

tissue. Stone et al. utilize cartilagenous tissue which clearly still exhibits porcine antigens that cause immune attack of the transplanted tissue. There is no teaching relating to the viability of the transplant tissue and no suggestion that the transplanted tissue retains the ability to re-express any temporarily removed surface antigens.

As seen from the Stone et al. reference, the harvested tissue was prepared for transplant by first immersing in alcohol for 5 minutes followed by immersion in a phosphate-citrate-sodium-chloride buffer containing 100 U/ml α-galactosidase for 4 hours at 26°C. (See, Materials and Methods, page 1578.) Applicant asserts that would be appreciated by a person of ordinary skill in the art that such treatment with alcohol would be lethal to the transplant tissue.

The Examiner's attention is directed to the Declaration pursuant to 37 C.F.R. §1.132 of Denise L. Faustman, M.D., Ph.D. submitted herewith. Dr. Faustman conducted experiments treating various eukaryotic cells by the same method taught in Stone et al. Specifically, cells were treated for 5 minutes in alcohol followed by incubation with 100 U/ml α-galactosidase in phosphate-citrate-sodium-chloride buffer for 4 hours at 26°C, and then the viability of the treated cell population was assessed. Dr. Faustman treated murine NOD and B6 T cells isolated from splenocytes, murine kidney cells, murine liver cells, and human peripheral blood lymphocytes (PBLs) according to Stone et al. as outlined above. As seen from the results shown in Table 4A, treatment of T cells isolated from splenocytes with alcohol alone for 5 minutes or alcohol for 5 minutes followed by 100U/ml α-galactosidase for 4 hours at 26°C, resulted in 100% cell death within 4 hours. The same results were seen with kidney and liver cells (Table 4B) and human PBLs (Table 4C) treated as described above. Treatment of cells in α-galactosidase alone (no alcohol) had no effect on cell viability.

Therefore, there is no disclosure in Stone et al. that cures the failure of either of the Oliver et al. patents alone or in combination with the Galati et al. publication to provide any teaching that approximates the method of the invention as defined by the present claims. Taken together, the combined references teach the person of ordinary skill in the art that transplant tissue must be exposed to sodium azide, formaldehyde, acetone or alcohol in lethal concentrations prior to transplantation *and* that is this is a requirement for a successful permanent transplant. This is the opposite of Applicant's method. Accordingly, reconsideration and withdrawal of the rejection of Claims 10 and 11 under 35 U.S.C. §103 are respectfully requested.

With respect to Applicant's earlier remarks distinguishing the Stone et al. reference from the present invention, the Examiner states,

> "[A]pplicant appear to argue that the reference by Stone teaches a permanent removal of alpha-gal epitopes but not a re-appearance of MHC Class I antigens after transplantation in order to 're-educate' host immune response . . . Yet, the claimed invention does require alpha-galactosidase treatment in combination with papain and it does not require 're-education' as argued." (See, final Office Action, page 14.)

Applicant believes the Examiner has misinterpreted the nature of the disclosed invention. In contrast to the Examiner's assertion, the claimed invention inherently involves immune "re-education" as defined in the specification. "Re-education" is a term of art defined by Applicant in the specification that leads to the inhibition of rejection of the donor tissue by the host organism, which is the crux of the present invention,

> "Since the tissues will remain viable after treatment according to this invention, expression of MHC molecules will continue, and eventually *reappearance of MHC antigens* on the donor tissue will occur, e.g., after transplantation . . . *Reappearance of MHC antigens* may be used to advantage for inducing tolerance of the donor graft, through *re-education of the recipient's immune system* to recognize and tolerate the donor antigens as they reappear." (See, Specification, page 5, line 32, to page 6, line 8, emphasis added.)

If the immune system of the host organism did not recognize the "temporarily ablated" and re-expressed MHC Class I surface antigens on the surface of the "viable" donor tissue, the host would immediately reject the donor tissue via attack by the host's immune system. Therefore, "re-education" is the mechanism whereby the host's immune system is "taught", by the returning expression of MHC Class I surface antigens of the donor tissue, to regard the donor tissue as "self" tissue.

In any event, the Examiner's argument is beside the point: It is not incumbent on Applicant to include in the claims a detailed explanation of the mechanism of immune "re-education" since it is already clear that the claims in their present form are distinguished from all the art of record and may be easily understood and practiced by those skilled in this art. No modification of the present claims is necessary to overcome the rejections of the final Office Action.

For the reasons set forth above, Applicant respectfully submits that the references cited by the Examiner in the present Office Action, considered together or individually, neither disclose nor suggest the novel method disclosed in the present application for inhibiting rejection of donor tissue transplanted into a recipient host. Reconsideration and withdrawal of the rejections are respectfully requested.

Reconsideration and allowance of the pending claims are respectfully solicited.

Respectfully submitted,

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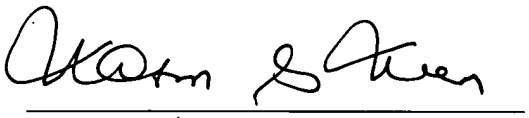

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